Antimicrobial activity of bark of *Moringa oleifera* L

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Abstract

Antimicrobial activity of different extracts of bark of *Moringa oleifera* was studied against ten bacterial strains These bacteria are both gram +ve and gram -ve. Bark were extracted with a petroleum ether, chloroform, ethyl acetate, ethanol and aqueous . In the present work the antibacterial activity was done by cup plate method. The antibacterial activity was expressed as zone diameter in millimeters. Different extracts from bark of the plant was compared with standards like benzyl penicillin for gram +ve bacteria and streptomycin for gram –ve bacteria using DMF as control. The readymade media for inoculum and culture was obtained from Himedia labs. Prepared herbal extracts from the bark of the plant were screened against bacteria organisms at the concentration range between 50 µg and 300 µg/0.1ml. The present investigation reveals that the aqueous, chloroform and ethyl acetate extracts and in some cases petroleum ether extract showed significant antimicrobial activity when compared with standard.

Key words:, Moringa species, antibacterial, soxhlet extraction, streptomycin, benzyl penicillin,

Introduction

Moringa oleifera belonging to family Moraginaceae is a small or medium tree about 9 to 12 m , high available in Himalayan region and cultivated all over the plains of India[1] . *M.oleifera* fruit is a hanging capsule opening on 3 sides, up to 1.2 m long and triangular with 9 ribs. The seeds are triangular, light brown to black, with 3 thin, whitish wings, approximately the size of a hazelnut[2]. *M.oleifera* is well known herb used in many pathological condition and mentioned in many ancient literature for its useful action in dysthria, appetizing, stomachic and heals ulcer. Fruits contain minerals, vitamins and amino acids. Plant also showing presence of quercetin, kaempferol. *Moringa oleifera* increase healing of gastric ulcers and also prevent the development of experimentally induced gastric ulcers and duodenal ulcers in rats[3].

Moringa oleifera Methanolic extract stimulate both humoral immune and cellular response. High dose is less effective than low dose[4]. *Moringa oleifera* possesses hypoglycemic activity in STZ induced diabetic Wistar rats only[5].The present study aimed evaluation of antimicrobial activity of bark extract of *Moringa oleifera* L.

Materials and Methods

Chemical, Media and Antobiotics

The organic solvents such as petroleum ether, chloroform, ethyl acetate, ethanol and DMF were obtained from Loba Chemical Pvt. Ltd., Mumbai. Nutrient broth, Nutrient agar were obtained from Himedia Laboratories Pvt. Ltd., Mumbai. Benzyl penicillin injection was obtained from IDPL and streptomycin sulphate from Sarabhai chemicals, India

Bark Collection

Preparations of plant extracts

The bark of *Moringa oleifera* were collected from local areas of Karjat, Maharashtra and authenticated from SMT. B.N.B. Swaminarayan Pharmacy College Salvav, Vapi.Gujrat. The air-dried bark of *Moringa oleifera* Linn. belonging to family moraginaceae were reduced to fine powder (40 size mesh) and around 100 gm of powder was subjected to successive hot continuous extraction (soxhlet) with petroleum ether, chloroform, ethanol and ethyl acetate. Another batch of powdered drug was macerated with

chloroform-water I.P. (Each time before extracting with next solvent the powdered material was dried at room temperature). By using rotary flash evaporator solvent were concentreated after effective extraction[6].

Antibacterial activity [7].

In the present work to know the antibacterial activity cup-plate method is employed. The antibacterial activity is expressed as zone diameter in millimeters, which is measured with a divider. Different extracts of bark of the plant was compared with standards and DiMethyl Formamide (DMF) as control for antimicrobial activity.

Standard

1.Benzyl penicillin for gram +ve bacteria

2.Streptomycin for gram-ve bacteria

Preparation of sample solution

Different concentration of extracts equivalent to 200 µg, 150 µg, 50 µg, 100 µg, and 300 µg/o.1ml by using DMF were prepared.

Preparation of standard solutions

Standard benzyl penicillin injection IP 1,00,000 units.

As per IP 1mg of benzyl penicillin=1500-1750 IU.

Benzyl penicillin injection (IP) 1,00,000 units manufactured by IDPL a streptomycin sulphate (ambistyn 1.0 gm) manufactured by Sarabhai chemicals were used. Different concentrations of standards equivalent to 50 µg, 100 µg, 150 µg, 200 µg and 300 µg/0.1ml of benzyl penicillin and streptomycin were prepared.

Preparation of inoculum

Nutrient agar medium (Himedia labs) of the following composition was used for preparation of slants.

Peptone----------5.0 gm
Beef extract----------1.5 gm
Sodium chloride----------5.0 gm
Agar----------15.0 gm
Yeast extract ----------15.0 gm
Distilled water to make ---1000 ml

About 28 gm of prepared medium was taken in 1000 ml distilled water and boiled to dissolve completely. Under aseptic condition microorganisms were streaked, and the

slants were incubated at 37±1ºC for 24 hrs. These 24 hrs cultures were used for preparation of inoculum. In 10ml sterile water microorganism suspension were prepared and to 100 ml 0.5 ml of this suspension was added of the agar medium.

Culture medium

In the present investigation antibiotic medium (nutrient agar –Himedia) was employed possessing the following composition (Ready made medium).

Peptone----------6.0 gm
Beef extract----------1.5 gm
Agar----------15.0 gm
Yeast extract –----------3.0 gm
Distilled water to make --1000 ml.

About 27 gm of above readymade medium was dissolved in freshly prepared distilled water (in 1000 ml) by gentle heating.

Preparation of agar plates

The sterilized medium was cooled at 40ºC and 0.5 ml of inoculum per 100 ml of medium was added in conical flask. To avoid the formation of air bubbles shaken gently and then transferred into petridishes so as to obtain 6 mm thickness of medium. The medium in the plate was allowed to solidify at room temperature.

Experimental procedure

The sterile borer was used to prepare 4 cups of 7 mm diameter in the medium of each petridish. An accurately measured 0.1 ml solution of each concentration of solution of extracts and standard samples were added to the cups in the medium by using micropipette. At room temperature micropipette kept for effecting diffusion of drug extracts and standards later they were incubated at 37±1ºC for 24 hrs. The presence of definite zones around the cup of any size indicated antibacterial activity. The control was run simultaneously to assess the activity of DMF, which was used as vehicle for extract and fractions. Finally zone of inhibition was measured interms of diameter.

Results and Discussion

Herbal extracts prepared from the bark of the plant were screened against ten bacterial strains and four fungal organisms for the purpose of in vitro qualitative evaluation in the concentration range between 50 µg and 300 µg/0.1ml.

Along with petroleum ether extract the ethanolic, ethyl acetate, chloroform and aqueous extracts were subjected for antimicrobial activity. In these extracts aqueous, ethyl acetate and chloroform extracts showed pronounced antibacterial (Table 1., 2 and Table 3).

In antibacterial activity both gram +ve and gram –ve organisms were used. For the gram +ve organisms like *Bacillus subtilis*, *Bacillus cerius, Staphylococcus aureus* the chloroform and ethyl acetate extracts showed significant anti bacterial activity at 50 µg/0.1ml, when compared with standard. For *Salmonella typhi,* the aqueous and ethyl acetate extracts showed minimum inhibitory concentration (MIC) at 100 µg/0.1ml.

For the gram –ve organisms like *Pseudomonas aerogenosa*, the aqueous and ethyl acetate extracts showed significant antibacterial activity at 50 µg/0.1ml, for *Escherichia coli,* the aqueous and chloroform has MIC at 50 µg/0.1ml, for *Klebsiella pneumonae,* the ethyl acetate and chloroform extracts has MIC at 300 µg/0.1ml, for *Vibrio cholerae,* the aqueous and chloroform has MIC at 300 µg/0.1ml, for *Proteus mirabilis,* the aqueous and chloroform has MIC at 300 µg/0.1ml,while for *Serratia marsupium* it was 50 µg/0.1ml, when compared with standard.

The present investigation reveals that the aqueous, chloroform and ethyl acetate extracts shows significant antibacterial activity when compared with standard.

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Antibacterial Activity of *Moringa oleifera*																				
Extracts	**Bacillus Subtilis**					**Bacillus Cerius**					**Staphylococcus aureus**					**Pseudo aerogenosa**				
	50	**100**	**150**	**200**	**300**	**50**	**100**	**150**	**200**	**300**	**50**	**100**	**150**	**200**	**300**	**50**	**100**	**150**	**200**	**300**
Ethanolic	8.5	9.5	10.5	11.4	12.6	9.4	10.3	10.9	11.3	11.8	8.6	9.8	10.5	11.3	11.5	9.3	10.5	11.6	12.1	12.4
Ethyl acetate	14.8*	16.5	18.6	20.5	22.8	12.8*	14.8	17.2	18.1	19.5	15.8*	17.5	19.6	21.4	23.1	13.5*	16.1	18.5	20.5	22.1
Chloroform	13.8*	15.3	17.1	18.8	21.5	13.4*	15.5	17.6	20.3	22.3	14.8*	16.2	17.8	19.5	21.3	13.3*	14.4	16.5	19.3	21.3
Pet ether	9.8	10.3	11.2	11.8	12.9	9.5	10.5	11.3	11.9	12.5	8.6	9.8	11.5	12.1	12.5	9.1	9.7	10.7	11.5	12.2
Aqueous	13.2*	14.2	16.1	17.5	20.5	13.8*	15.3	17.9	20.3	22.1	13.5*	15.3	16.6	18.8	21.5	14.5*	16.5	19.1	20.6	24.5
DMF	R	R	R	7.7	7.8	R	R	R	7.8	7.9	R	R	R	7.8	7.9	R	R	R	7.5	7.7
STD	16.2	20.1	21.7	14.2	27.3	16.3	20.3	21.8	24.3	28.3	15.8	19.6	22.8	25.3	27.3	15.5	18.6	21.7	24.8	26.6

Diameter of cup (7mm)
Average of 3 readings
Reagings are in millimeter (mm)

Antibacterial Activity of *Moringa oleifera*																				
Extracts	**Salmonella typhi**					**E. Coli**					**Klebsiella Pneumnae**					**Vibrio-cholerae**				
	50	**100**	**150**	**200**	**300**	**50**	**100**	**150**	**200**	**300**	**50**	**100**	**150**	**200**	**300**	**50**	**100**	**150**	**200**	**300**
Ethanolic	8.1	9.1	9.7	10.5	11.5	8.3	9.1	9.7	10.5	11.5	8.5	9.2	9.9	10.6	11.4	8.9	9.7	10.6	11.5	12.1
Ethyl acetate	13.6	15.5*	18.2	19.6	21.6	14.2*	15.6	17.6	19.8	21.6	13.7	15.7	18.6	21.7	23.8*	13.1	15.1	17.3	19.1	21.7*
Chloroform	14.5	16.6*	18.6	20.6	21.5	14.4*	16.5	18.5	20.5	22.6	11.8	14.8	17.6	19.7	22.1*	13.4	15.8	18.6	21.1	22.8*
Pet ether	8.9	9.6	10.5	11.4	12.2	9.1	9.6	10.2	10.6	11.1	8.5	9.8	10.2	10.8	11.5	9.1	9.8	10.9	11.4	12.1
Aqueous	14.2	15.2*	16.6	19.8	22.6	13.8*	15.4	17.6	19.6	22.4	13.5	15.4	17.1	18.5	20.8*	14.4	16.9	18.8	21.4	23.6*
DMF	R	R	R	7.7	7.8	R	R	R	7.7	8	R	R	R	7.7	7.9	R	R	R	7.7	7.9
STD	16.5	17.8	20.5	22.8	24.9	15.5	17.8	19.8	22.6	24.8	17.3	19.5	21.5	23.1	24.5	16.8	18.6	20.5	22.7	24.5

Diameter of cup 7mm
Average of 3 readings
Reagings are in millimeter (mm)

Antibacterial Activity of *Moringa oleifera*										
	Proteus mirabilis					**Serratia marsupium**				
	50	**100**	**150**	**200**	**300**	**50**	**100**	**150**	**200**	**300**
Ethanolic	8.3	9.3	10.5	11.7	12.1	8.5	9.2	9.7	10.8	11.7
Ethyl acetate	12.8	14.9	16.9	19.1	21.7*	13.4*	15.6	17.7	19.3	22.4
Chloroform	13.8	15.7	18.1	20.5	23.1*	13.6*	15.6	18.2	19.8	22.1
Pet ether	9.1	9.5	10.4	11.1	11.6	8.5	9.8	12.1	13	13.9
Aqueous	13.9	15.8	17.9	19.9	23*	13.5*	14.8	18.1	20	21.9
DMF	R	R	R	7.5	7.7	R	R	R	7.5	7.7
STD	18.5	20.5	22.1	22.5	24.5	17.4	19.6	21.7	23.6	27.5

Diameter of cup
7mm)
Average of 3
readings
Reagings are in millimeter (mm)